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CELSS

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I. INTRODUCTION

During the past year, we have studied several aspects of the continuous culture of the green alga Scenedesmus obliquus (Gaffron strain D_3). Our primary goals were to 1) set up and maintain continuous cultures, 2) monitor the cultures to determine culture stability, biomass production, and by-product production, and 3) determine the efficiency of nitrogen utilization and the possible production of nitrogen by-products, such as N_2O.

This annual report is basically an update and expansion of material presented earlier (Progress Report, Nov. 1981; Renewal Proposal, Mar. 1982). We have taken this opportunity to present our work in much greater detail.

II. DESCRIPTION OF THE CONSTANT CELL DENSITY APPARATUS (CCDA) FOR CONTINUOUS CULTURE OF ALGAE

One of the primary components of the Martin Marietta Laboratories' CELSS algal culture studies is the maintenance of continuous cultures. The design of our continuous culture system was derived from the earlier work of several other scientists(1-3) and we believe it represents a summation of their best ideas cast in terms of state-of-the-art technology. A primary goal of the design and construction of these CCDA's was to provide a means to control and monitor important photosynthetic parameters, such as light flux, light absorption, and temperature. Another important consideration was our goal of constructing a system that was harvestable on demand. This latter requirement precluded the use of a chemostat system; instead, we use the turbidostat system described below.

Figures 1 and 2 are exploded and assembled diagrams of the CCDA constructed and used in our laboratory. Figure 3 is a photograph of the assembled operating system. As is evident from Fig. 1, the system is of modular construction for easy maintenance and repair. Components in contact with the algae are made of machinable polycarbonate plastic or Viton, both of which can be sterilized by autoclaving or by rinsing with ethanol. According to the results of preliminary tests using Scenedesmus and Chlorella, neither material caused toxic or other undesirable effects (e.g., cell adhesion). These results agree with earlier findings.[4]

The culture system consists of three concentric, cylindrical, transparent chambers. The middle chamber, which contains the algal culture, has a volume of about 675 ml and is sandwiched between two water jackets that contain constant-temperature water. Temperature control

is provided by a refrigerated bath circulator (Neslab RTE-4; +0.01°C temperature control from -30° to +100°C).

The cells are illuminated from inside the innermost cylinder. We are currently using Sylvania Grow-lux fluorescent bulbs, which provide much more red light than standard fluorescent bulbs. Intensity can be adjusted by choice of lamp (40W to 215W) and use of neutral density filters.

Cell density is maintained by monitoring the light transmission through the culture using a photoconductive cell (Clairex CL604L). The output of this photocell is amplified, integrated (to remove the ac component from the light), and compared to a reference voltage. When the processed photocell output differs sufficiently from that of the reference for several seconds (we currently use 4 s), a modified Teflon solenoid valve (Valcor 51C56T34-ID) is triggered to admit a preselected amount of nutrient (currently about 6 ml) to the culture. After a latent time (currently 40 s) to allow for mixing, the monitoring cycle is reinitiated.

This monitoring system is located near the bottom of the chamber, and thus it remains effective even after a substantial percentage of the algae have been harvested. During normal untended operation, algae are automatically bled off via an overflow system when the culture chamber is filled.

Gas (either air or CO_2-enriched air) is admitted through the bottom of the culture module at a flow rate of about 500 cm^3/min, which is sufficient to provide relatively rapid mixing and prevent cell settling. In this respect the apparatus closely resembles an air-lift fermenter.

III. CCDA OPERATING CHARACTERISTICS

During this contract period, we have been growing algae in two of the culture modules described in Section II. Except for the nitrogen source (NO_3^- vs urea), growth conditions were identical, i.e., temperature, 30°C; 1% CO_2 in air; illumination, 110-W fluorescent growth lamp, 130-150 $\mu E\ m^{-2}\ s^{-1}$ photosynthetically active radiation (PAR) at the external surface; and a cell density equivalent to ~ 100 μg chl ml^{-1}, which resulted in PAR absorption of 90 to 95%. The cultures were monitored daily for packed cell volume (PCV), chlorophyll, dry weight, reproduction rate, and pH, and intermittently for glycolate, total N, and microbial contamination (see Appendix A).

We maintained one of these cultures for about 4 months without interruption (both were finally shut down at the end of December). The operating data collected during these runs are shown in Figs. 4 and 5 and Tables 1 and 2. During this time we found that the major cause of culture instability was opto-electrical, not (directly) biological. That is, most of the decrease in biomass shown in the figures and tables reflects changes in apparent turbidity (cell density) due to wall growth and the formation of small "hills" from settled algae. These problems were subsequently alleviated by 1) raising the detector, and 2) lowering the pH of the medium (wall growth seems to be favored by high pH). These slow, long-term drifts can be eliminated (and indeed were in subsequent runs) by changing the photocell reference voltage to bring the chlorophyll and dry weight values back in line.

One of the primary concerns with respect to a CELSS is the energy efficiency of the biological food production/air regeneration system. Although this was not our primary concern during the past year, our results are encouraging. Our data suggest that the quantum yield (400 - 700 nm) for algal growth in our unoptimized apparatus is ~ 0.056, which is about half the maximum theoretical quantum yield for photosynthesis. Table 3 shows representative data and calculations.

We addressed the interrelated topics of algal by-product excretion and microbial contamination by periodically assaying the culture supernatant. To date, we have detected no (< 1 ppm) glycolate (the primary algal excretory product) while our cultures are in the steady state. Parallel microbial assays indicated a low (0.1 - 0.01%) non-algal biomass that did not change appreciably (with time) with respect to amount or species composition. These findings suggest that microbial contamination should not be a significant problem in a CELSS since 1) the algae seem to excrete little or no organic compounds, and 2) microbial populations, even when present, do not take over the culture.

IV. EXCRETION OF ALGAL BY-PRODUCTS

As already described (Section III), we have periodically assayed the culture supernatant for the presence of glycolate. To date, none has been detected (detection limit ~ 1 ppm) in cultures grown on air, 1% CO_2-air, or 2.5% CO_2-air. This result confirms the experience of J. Myers (Univ. of Texas) who "never detected glycolate in happily growing cultures." However, our data did not rule out the transient production of glycolate due to changes in CO_2 tension or excretion due to environmental stress. Indeed, it has been reported[5] that high-CO_2-grown algae can excrete substantial amounts of glycolate when illuminated under CO_2-limiting conditions, although a substantial lag (~ 15 min) can occur before they do so.[6] Since these algae can adapt to low CO_2 in 60 to 90 min,[7] glycolate excretion by this mode may be a rather transient phenomenon.

In light of these prior reports, we undertook a series of experiments to determine the amount of glycolate excreted by algae after a high CO_2-to-air transition. These experiments also included parallel COD (chemical oxygen demand) assays as a measure of the total organic carbon in the supernatant. Although our experiments to date have been rather disappointing in terms of precision and reproducibility, we can at least provisionally conclude that gylcolate and other organic carbon excretion is not a major process (with respect to the total culture) after a CO_2-to-air transition. [Our cultures fix ~ 50 mg C in 1 hour. If all this C (admittedly an extreme case) were to be converted to glycolate, this would be equivalent to ~ 220 ppm glycolate accumulated in one hour. Our observed values are about one-hundredfold less than this.]

V. NITROGEN UTILIZATION AND EXCRETION

Krauss and collaborators have reported that a significant fraction of the NO_3^- nitrogen provided to Chlorella cultured in a Recyclostat was lost, probably as N_2O.[8] The release of this gas into the atmosphere of a CELSS could cause grave problems for the air regeneration system, as well as contribute to a lack of closure of the nitrogen cycle.

Several years ago, we observed the production of trace quantities of N_2O by illuminated Scenedesmus[9] and the transient production of N_2O by soils during the process of denitrification,[10] both probably due to a side reaction during the course of NO_3^- reduction. The data of Krauss may reflect a similar process. His algal cultures were maintained under strong light, and often at high temperatures (39°C), conditions that may favor N_2O evolution during the course of NO_3^- reduction.

Because of these earlier results, one of the primary goals of the current project is to determine the nitrogen balance of our algal cultures, and specifically, whether compounds such as N_2O are excreted into the medium. Our initial approach has been to determine the nitrogen levels of the nutrient medium, cell-free efflux, and the harvested algae (Appendix B).

Table 4 shows the results obtained for NO_3^--grown cultures when the nutrient medium, culture supernatant, and algae were monitored for N, C, and H by an elemental analyzer. Although the precision is not optimal (probably due to the procedures used to dry the supernatant), there is no indication of N loss from these experiments.

Table 5 shows the results of a similar experiment in which the supernatant and nutrient medium were analyzed by ion chromatography and

the algae analyzed by an outside laboratory. The results of this experiment show surprisingly good agreement between added and recovered N.*

The results of these experiments suggest that the nitrogen entering the culture (as NO_3^-) was either incorporated in the algae or appeared as NO_3^- in the efflux supernatant. We have had no indication to date that the nitrogen is lost by the system. Although these results do not prove that there is no nitrogen loss from the system, they do suggest that any loss must be small (e.g., < 1%), at least for NO_3^-. These findings contradict those of Krauss, who found substantial (~ 20%) nitrogen loss in his culture system. The difference probably reflects variations in:

1) Light intensity. The Krauss system used very high light intensity, well beyond that required to saturate photosynthesis.

2) Algal species. Scenedesmus, much more so than Chlorella, is able to reduce O_2, and thus provides a means to safely dispose of excess reductant (see March 1980 proposal).

Because our data obtained with Scenedesmus point to a very low production of nitrogenous by-products, we have not attempted to determine N_2O directly. (Current data indicate that the N_2O concentration in the gas stream would be too low to monitor directly.) We have observed only traces of NO_2^- (< 1 ppm) in the effluent supernatant.

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* Although not germane to the present discussion, note that $PO_4^{\equiv}$ analyses also showed good recovery.

We have also undertaken similar experiments with urea-grown cultures. Although results from these experiments point to a less-than-qualitative recovery of urea-N, we have been unable to demonstrate that this loss of N is alga-mediated; indeed, we suspect that it isn't. Urea is fairly unstable in solution (e.g., it cannot be autoclaved) and thus recovery experiments will be -- and indeed have been -- compromised by the loss of urea-N as NH_3. (There may also be extracellular urease activity in our cultures, although we have not checked this.)

VI. FINALE

This Annual Report is also an interim report and consequently, much of the described work is still in progress. During the coming contract year, we will strive to conclude the by-product excretion studies and nitrogen utilization described in Sections IV and V. We will also initiate studies on CO_2 and light utilization (see Martin Marietta Laboratories Proposal No. BI 82-351, March 1982). In these latter studies we will attempt to identify the capabilities, limitations, and tradeoffs relevant to algal culture in a CELSS.

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TABLE 1

Data Obtained During Continuous Culture of Scenedesmus with Urea

Date (1981)	pH	PCV (μl/ml)	Chl. (μg/ml)	Reproduction (ml/hr)	Dry Weight (mg/ml)	Cell Counts (number cells/ml)	Contamination (number bact/ml)	Density Reference and Other Notes
Aug 20	6.25	9.0	88	52.4	2.4			1.965
21	6.25	9.0	90	54.8				
24	6.25	8.5	88		2.28	$8.0x10^7$		
25	6.15	9.0	84	55.8	2.38			
26	6.15	8.5	85	58.2	2.32			
27	5.9	8.0	79	59.4	2.32			
28	6.0	8.0	88	55.4	2.4			
31	5.9	8.0	80		2.16	$8.0x10^7$		
Sep 1	5.9	8.0	84	57.5	2.21			
2	5.95	9.5	120	50.0	2.63			
3	5.95	9.0	109	57.5	2.43	$9.5x10^7$		
4	5.9	10.0	112	49.4	2.76			
8	6.0	9.5	111		2.54		$2.92x10^6$	
9	6.0	10	115		2.72			
10	6.8	9.5		49.1	2.55	$8.95x10^7$		
11	6.0	10	120		2.85			
14	6.1	10	109		2.64		$2.25x10^6$	
15	6.1	10	109	46.5	2.57			
16	6.0	10	102		2.55			
17	5.95	13.5	133		2.98			
18	6.0	11.0	123	49.4	2.79			
21	5.5	12.0	107		2.69	$7.26x10^7$	$2.6x10^6$	
22	5.7	11.0	107	40.0	2.92			
23	5.7	10	104					
24	5.55	11.0	104	50.7	2.72			
25	5.65	10.0	104	46.5	2.86			
28	5.65	8.5	112		2.48	$8.65x10^7$		
29	5.4	8.5	98	50	2.46			
30	5.3	8.5	99	51.9				

Table 1. (Continued.)

Date (1981)	pH	PCV (µl/ml)	Chl. (µg/ml)	Reproduction (ml/hr)	Dry Weight (mg/ml)	Cell Counts (number cells/ml)	Contamination (number bact/ml)	Density Reference and Other Notes
Oct 1	5.4	9.0	103	51.9				
2	5.3	8.0	104					
5	5.5	7.5	96		2.28			
6	5.45	7.5	104	50	2.25		$1.2x10^6$	
7	5.3	7.5	96	52.5	2.29	$6.75x10^7$		2.110
8	5.4	8.0	118	53.8	2.36			
9	5.25	8.0	120	47.9	2.21			
12	5.8	8.5	118		2.23			
13	5.7	8.0	104	52.2	2.23			
14	5.7	8.0	115	50.0	2.23			
15	5.8	8.5	117	45.6	2.21			
16					2.30			
19	5.7	8.0	109		2.28			
20	5.5	8.5	115	42.5	2.27			
21	5.6	9.5	117	39.4	2.20			
22	5.8	9.5	124	45.0	2.30			
23	5.7	9.0	115	46.9				
26	5.75	8.0	110		2.40			
27	5.7	8.0	101	47.2	2.15			
28	5.5	7.5	110	48.8	2.25			
29	5.2	7.5	103	48.8	2.15			
30	5.3	7.5	112	48.1	2.24	$6.09x10^7$		
Nov 2	5.6	8.0	117		2.13		$8.47x10^5$	
3	5.65	8.0	112	48.1	2.22			
4	5.75	7.5	115	50.0	2.14			
5	5.8				2.17			
6		8.0	109		2.23		$7.3x10^5$	
9	5.8	12.5	190		3.05			
10	5.85	7.0	107	46.9	2.02			
11	6.0	7.5	109	41.2	2.05			
12	5.85	7.0	104	46.5	2.07			
13	5.75	8.0	107	45.2	2.16		$2.47x10^5$	

Table 1. (Continued.)

Date (1981)	pH	PCV (μl/ml)	Chl. (μg/ml)	Reproduction (ml/hr)	Dry Weight (mg/ml)	Cell Counts (number cells/ml)	Contamination (number bact/ml)	Density Reference and Other Notes
Nov 16	6.1	7.0	107		2.01			
17	6.0	7.0	107	46.7	1.98			
18	5.8	7.0	109	50.3	2.05			
19	5.7	7.5	112	48.5	2.14			
20					2.05	7.9×10^7		
23								
24	5.7	7.5	116	43.8	2.21			
25	5.7	7.5	112	44.4	2.12			
30	5.5	7.0	112		2.11		1.85×10^6	
Dec 1	5.4	7.0	110	45.5	2.09	7.78×10^7		
2	5.5	7.0	104	48.1	2.00			
3	5.4	7.0	110	42.5	2.01			
4	5.4	6.0	96	45.3	1.92			
7	5.6	6.5	96		1.92			
8	5.6	7.0	107	43	2.01		2.53×10^6	
9	5.6	7.0	113	37.5	2.09			
10	6.0	6.5	96	43.9	1.93		2.06×10^6	
14	5.8	6.5	100		1.85			
15	5.9	6.5	104	50	1.85			
16	5.8	6.5	104	44.2	1.83			
17	5.7	6.0	98	46.9				
21	6.1	6.5	96					

TABLE 2

Data Obtained During Continuous Culture of Scenedesmus with NO_3^-

Date (1981)	pH	PCV (µl/ml)	Chl. (µg/ml)	Reproduction (ml/hr)	Dry Weight (mg/ml)	Cell Counts (number cells/ml)	Contamination (number bact/ml)	Density Reference and Other Notes
Aug 20	7.7	8.0	57	58.9	1.93			2.14
21	7.75	7.0	62	67.9				
24	7.7	7.0	54		1.85	4.15×10^7		
25	7.65	7.0	56	62.4	1.78			
26	7.7	7.0	57	61.8	1.74			
27	7.7	7.0	59	58.7	1.57			KNO_3 increased to 20 mM
28	7.7	6.0	61	65.8	1.44			
31	7.6	5.0	52		1.2	2.3×10^7		
Sep 1	7.5	5.0	49	71.9	1.23			
2	7.7	8.0	91	58.1	1.81			
3	7.8	9.0	109	51.3	2.02	3.35×10^7		
4	7.65	7.0	79	61.3	1.69			
8	7.7	6.5	86		1.66		4.05×10^6	
9	7.65	7.0	86		1.66			
10	7.5	6.5		55.4	1.61	3.49×10^7		
11	7.8	6.0	107		1.70			2.32
14	7.8	6.0	90		1.71		3.53×10^6	
15	7.75	7.0	91	56.1	1.77			
16	7.7	7.0	85		1.69			
17	7.8	7.0	91		1.64			
18	7.75	7.0	86	48.2	1.8			
21	7.6	7.0	94		1.83	4.73×10^7	5.26×10^6	
22	7.8	7.0	94	53.1	1.77			
23	7.65	7.0	87					2.48
24	7.65	7.0	96	53.3	1.77			
25	7.7	7.0	94	55.3	1.84			
28	7.0	7.0	101		1.74	4.67×10^7		
29	7.4	6.0	89	61.9	1.72			
30	7.3	6.0	83	61.3				

Table 2. (Continued).

Date (1981)		pH	PCV (µl/ml)	Chl. (µg/ml)	Reproduction (ml/hr)	Dry Weight (mg/ml)	Cell Counts (number cells/ml)	Contamination (number bact/ml)	Density Reference and Other Notes
Oct	1	7.4	6.0	83	59.4			6.65×10^6	
	2	7.4	6.0	78					
	5	7.3	6.0	78		1.52			
	6	7.2	6.0	78	54.4	1.50		6.67×10^6	
	7	7.0	6.5	78	60.6	1.52	3.8×10^7		2.600
	8	7.3	6.0	78	56.3	1.54			
	9	6.4	6.0	78	60.0	1.42			
	12	5.5	4.5	43		1.20			
	13	5.5	4.0	43	52.2	1.14			
	14	5.25	3.5	35	51.3				
				CLEANED AND RESTARTED → SAME CONDITIONS					
	23	7.4	7.0	92	45.1				2.70
	26	7.5	7.5	104		2.13			
	27	7.3	8.0	112	47.9	2.13			
	28	7.3	8.0	117	47.5	2.15	8.25×10^7		
	29	7.0	8.0	103	48.8	1.99			
	30	6.6	7.5	110	48.8	1.91	6.25×10^7	2.73×10^6	
Nov	2	6.2	7.5	110		1.90			
	3	6.3	8.0	107	48.8	1.96			
	4	6.4	9.0	117	47.5	1.99			
	5					1.90			
	6	7.8	9.0	105		1.89		4.2×10^6	
	9	6.6	9.0	110		1.98			
	10	6.4	9.0	96	43.1	1.85			
	11	6.6	8.0	130	40.6	1.86			
	12	6.45	8.0	101	49.0	1.74			
	13	6.7	8.0	99	49.0	1.87		6.33×10^6	
	16	6.55	8.0	106		1.86			

Table 2. (Continued).

Date (1981)	pH	PCV (μl/ml)	Chl. (μg/ml)	Reproduction (ml/hr)	Dry Weight (mg/ml)	Cell Counts (number cells/ml)	Contamination (number bact/ml)	Density Reference and Other Notes
Nov 17	6.7	8.0	115	46.7	1.88			
18	6.7	8.0	112	47.9	1.96			
19	6.5	8.0	96	50.9	1.82			
20					1.85	5.52×10^7		
23	6.4	8.0	114		1.84			
24	6.4	8.0	116	50.0	1.95			
25	6.3	8.0	120	46.9	1.93			
30	6.4	8.0	115		1.94		2.17×10^6	
Dec 1	6.3	8.0	113	47.3	1.85	5.62×10^7		
2	6.3	7.0	114	48.8	1.84			
3	6.2	8.0	104	49.4	1.84			
4	6.1	7.5	98	48.5	1.75			
7	6.0	7.0	100		1.75			
8	6.2	7.5	107	45.5	1.77		5.51×10^6	
9	6.3	7.5	113	43.5	1.81			
10	6.0	6.5	98	49.0	1.75		1.01×10^7	
14	5.7	7.0	98		1.77			
15	6.2	7.0	100	46.3	1.74			
16	6.3	7.0	107	44.8	1.76			
17	5.6	6.5	98	46.3	1.76			
21	6.3	7.5	99	49.5				

TABLE 3

Efficiency Calculations on Data of 22 January 1982

Measured Parameters

N Source	Light ($\mu E\ m^{-2}s^{-1}$) Incident	Absorbed	Production (ml/hr)	Dry Weight (mg/ml)
urea	155	147	38.6	2.9
NO_3^-	138	121	48.3	2.2

Working volume = 530 ml; Area = 0.17 m^2
Cells 50% C

For urea-grown culture:

(38.6 ml/hr) (2.9mg/ml) = 112 mg/hr

This is 56 mg C/hr or 28.2×10^{20} molecules C/Hr

Absorbed light: (147) (6×10^{17})$h\nu\ m^{-2}s^{-1}$ or

$5.4 \times 10^{22}\ h\nu$ culture^{-1} hr^{-1}

Quantum requirement: (540×10^{20})/(28.2×10^{20}) = 19 $h\nu$/C

A similar calculation for the NO_3^--grown culture gives a value of 17 $h\nu$/C.

Average quantum yield = 1/18 or 0.056

TABLE 4

Elemental Analysis of Algal Cultures

Residue, supernatant: 0.1415 g/50 ml
Residue, nutrient: 0.1133 g/50 ml
Algae dry weight: 0.08275 g/50 ml culture

Sample	% N	% C	% H
Supernatant	3.11	0.35	0.12
Nutrient	7.43	0.10	0.15
Algae	9.65	52.09	7.10

Calculations

NO_3^- Nutrient

N 0.1415 g (0.0743) = 0.01051 g
C 0.1415 g (0.001) = 0.00014 g
H 0.1415 g (0.0015) = 0.00021 g

NO_3^--Grown Algae

N 0.08275 g (0.0965) = 0.00799 g
C 0.08275 g (0.5200) = 0.04310 g
H 0.08275 g (0.0710) = 0.00059 g

NO_3^- Supernatant

N 0.1133 g (0.0311) = 0.00352 g
C 0.1133 g (0.0035) = 0.00040 g
H 0.1133 g (0.0012) = 0.00014 g

N Summation: Supernatant 0.00352 g
Algae 0.00799 g
0.01151 g

Analyzed nutrient 0.01051 g

$$\frac{0.01151}{0.01051} = 109.5\% \text{ recovery}$$

TABLE 5

Elemental Analysis of Algal Cultures Using Ion Chromatography

1) Ion Chromatography Nutrient Analysis (3 separate samplings)

NO_3^-:

1295 ppm

1280 ppm

1280 ppm

Average NO_3^- nutrient measured 1285 ppm or 1.285 g/l adjusting for volume change (for pH adjustment)

$$1.285 \times \frac{15.02}{15.00} = 1.287 \text{ g/l } NO_3^- \text{ nutrient}$$

$$\text{N is } \frac{0.1386}{0.6133} \; (NO_3^-)$$

$$\therefore \frac{0.1386}{0.6133} (1.287) = 0.291 \text{ g/l N in nutrient}$$

2) Ion Chromatography Supernatant Analysis (3 separate samplings)

NO_3^-:

530 ppm

545 ppm

540 ppm

Average NO_3^- measured 538 ppm or 0.538 g/l adjusting for volume change (for pH adjustment)

$$0.538 \times \frac{15.015}{15.00} = 0.539 \text{ g/l } NO_3^- \text{ supernatant}$$

$$\text{N is } \frac{0.1386}{0.6133} \; (NO_3^-)$$

$$\therefore \frac{0.1386}{0.6133} (0.539) = 0.122 \text{ g/l N in supernatant}$$

Table 5. (Continued)

3) Dry Weights (3 separate samplings)

1.885 g/l
1.880 g/l
1.845 g/l

Average dry weight 1.870 g/l

4) Galbraith Analysis: (Duplicate)

N 9.04%
9.11%

Average N 9.075% (9.08)

5) N by weight = (1.870 g/l) (0.0908) = 0.170 g N =

= 0.170 g N in algae suspended/l

6) N (nutrient - supernatant)

(0.291 - 0.122)g/l - 0.169 g/l N difference

N Algae 0.170 g

$$\frac{\text{N Algae}}{\text{N(Nutr. - Super.)}} \quad \frac{0.170 \text{ g/l}}{0.169 \text{ g/l}} \quad 100.6\%$$

7) Ion Chromatography Nutrient Analysis

$PO_4^{\equiv}$

79.0 ppm
79.0 ppm
79.0 ppm

$PO_4^{\equiv}$ Average 79.0 ppm measured nutrient
Volume adjustment (pH adjustment)

$$79.0 \text{ ppm} \times \frac{15.02}{15.00} = 79.1 \text{ ppm}$$

$P = 0.326\ (PO_4^{\equiv})$

$= 0.326\ (79.1)$

$= 25.8$ ppm = 0.0258 g/l P nutrient

Table 5. (Continued).

8) Ion Chromatography Supernatant Analysis

$PO_4^{\equiv}$

6.0 ppm
6.0 ppm
6.0 ppm

$PO_4^{\equiv}$ Average 6.0 ppm measured supernatant
Volume adjustment (pH adjusted)

$$6.0 \text{ ppm} \times \frac{15.015}{15.0} = 6.01 \text{ ppm}$$

$P = 0.326 \; (PO_4^{\equiv})$

$= 0.326 \; (6.01)$

$= 1.96$ ppm $\approx$ 0.002 g/l P supernatant

9) Average dry weight of algae 1.87 g/l

From Galbraith

Phosphorus 1.36%

P algae = (1.87 g/l) (0.0136) = 0.0254 g P/algae in 1 liter

P (nutrient - supernatant)

(0.0258 - 0.002) g/l = 0.0238 g/l

P algae = 0.0254 g/algae in 1 liter

$$\frac{\text{P algae}}{\text{P(nutr. - super.)}} = \frac{0.0254 \text{ g/l}}{0.0238 \text{ g/l}} = 106.7\%$$

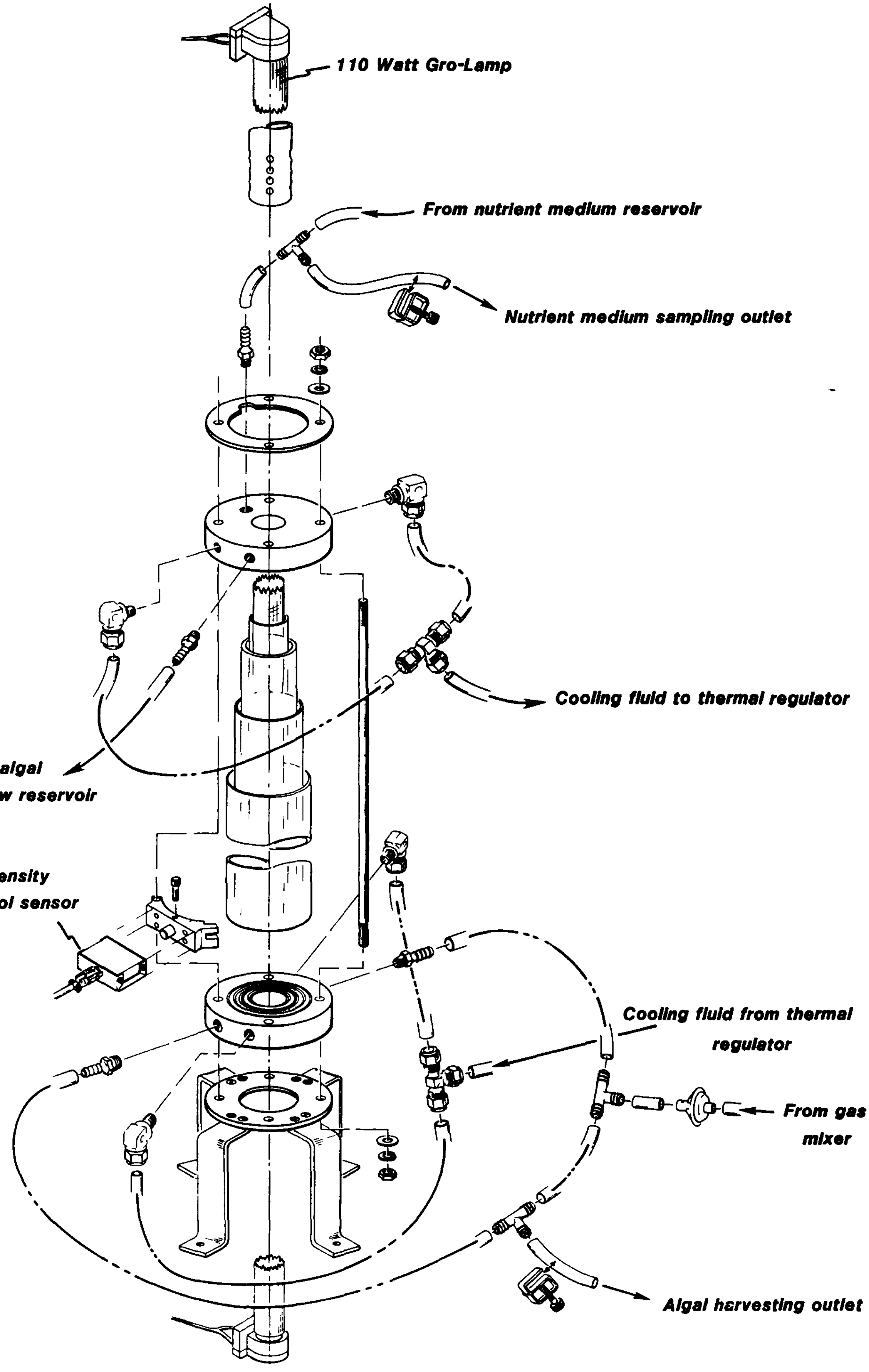

Figure 1. Exploded diagram of constant cell density apparatus (CCDA).

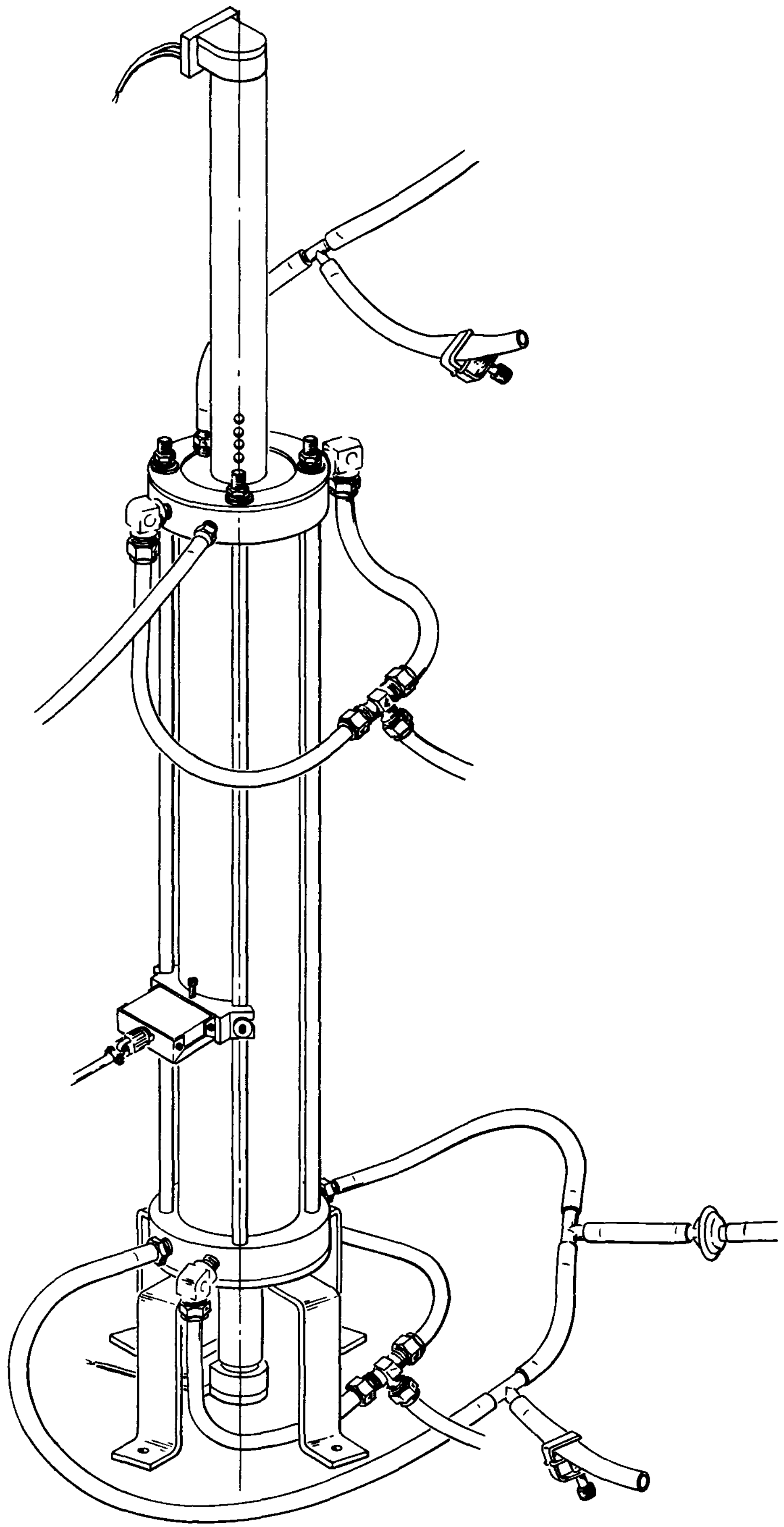

Figure 2. Diagram of assembled constant cell density apparatus (CCDA).

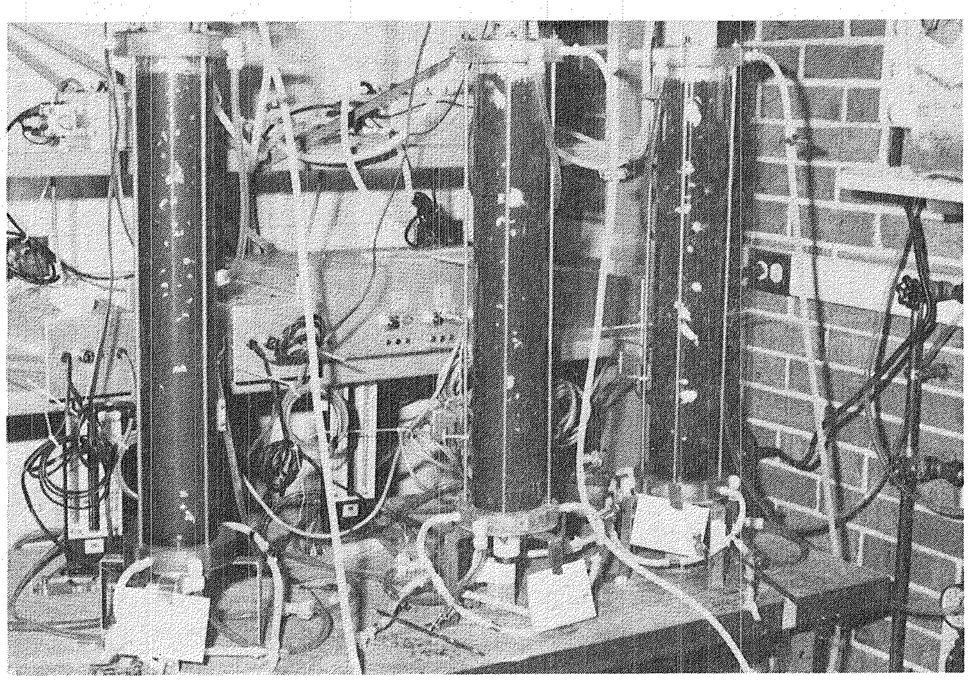

Figure 3. Photograph of operating constant cell density apparatus (CCDA).

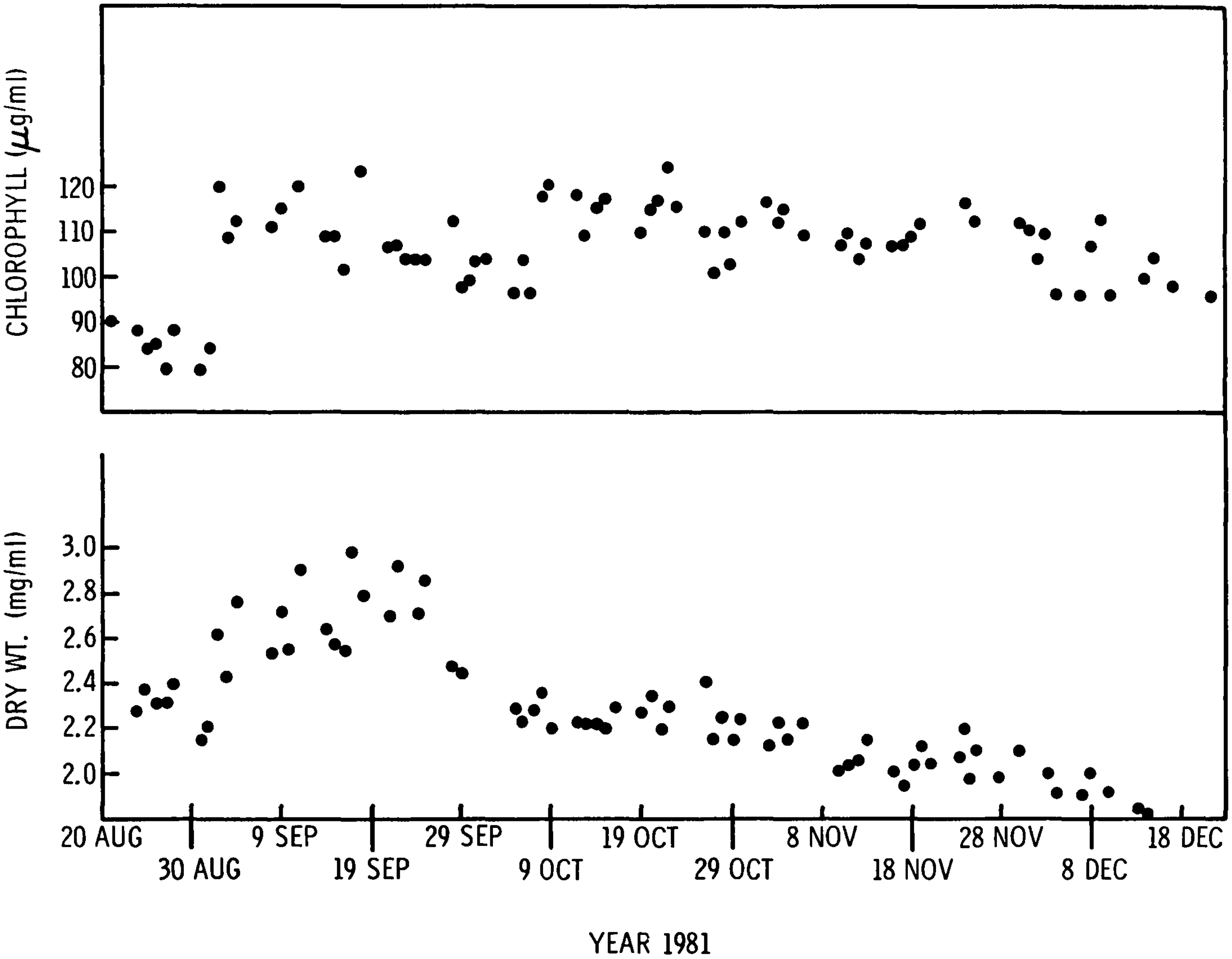

Figure 4. Data obtained during continuous culture of <u>Scenedesmus</u> with urea.

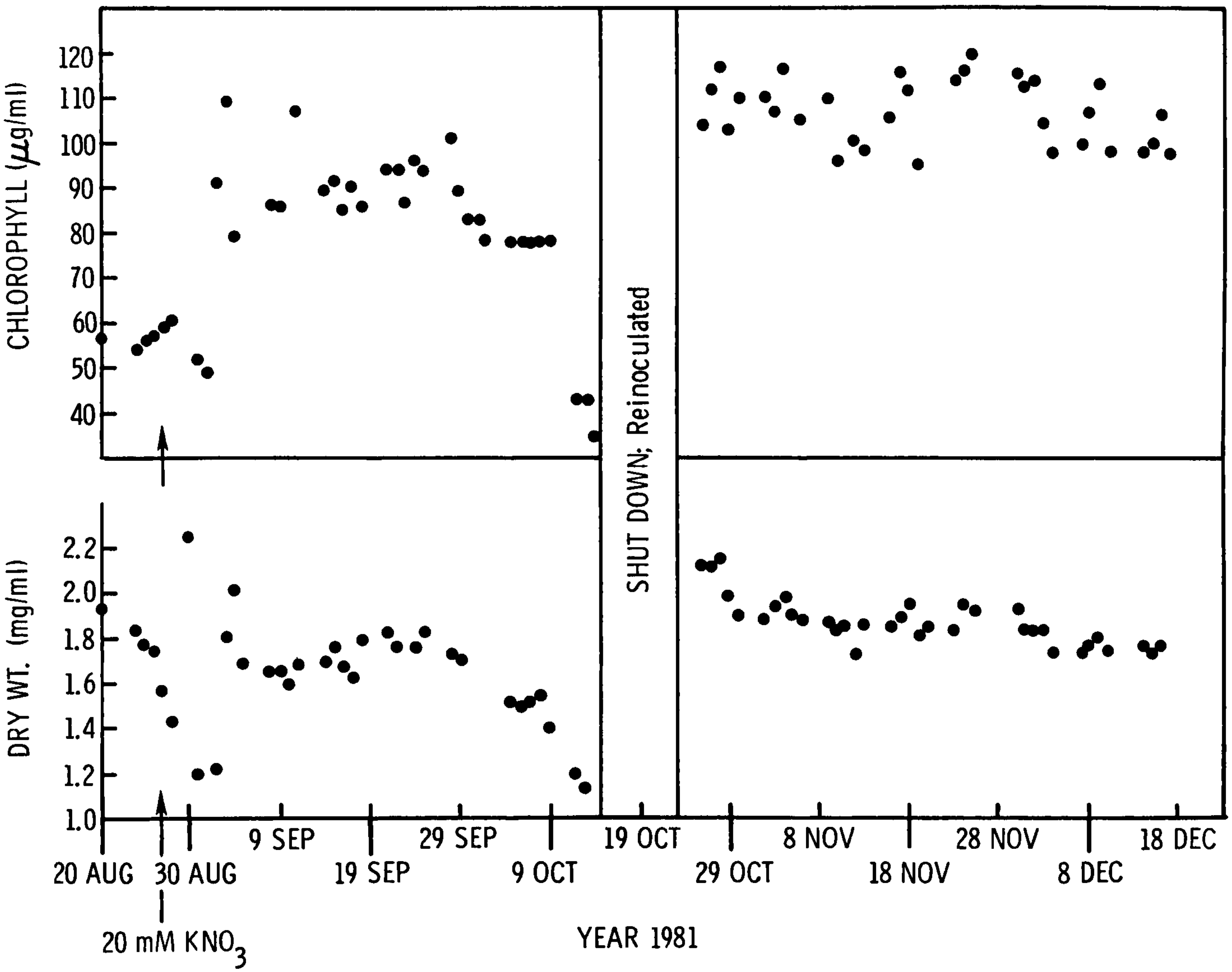

Figure 5. Data obtained during continuous culture of Scenedesmus with NO_3^-.

APPENDIX A

ANALYTICAL PROCEDURES

Packed Cell Volume (PCV)

One ml of the algal suspension was transferred to a modified hematocrit and centrifuged (in a clinical centrifuge, high speed) to a constant volume.

Chlorophyll

Aliquots of the algal culture were added to a 1:1 mixture of Triton X-100 and 5% KOH in MeOH, placed in a hot water bath at 63°C for 3 minutes, and centrifuged. Optical density was measured at 645 nm.

Reproduction

The overflow rate of the CCDA culture was determined by measuring the volume (ml) that overflowed over approximately a 16-hour period (overnight).

Dry Weight

Ten-ml aliquots were collected from the CCDA cultures via the sampling valve. The samples were filtered through glassine fiber paper (approx. retention 2.6 μm), rinsed thoroughly with distilled water, and dried to a constant weight at 110°C. (The filter paper was dried overnight at the same temperature prior to weighing; all samples were cooled to room temperature in a dessicator before weighing.)

Cell Counts

A leucocyte counter (1/16 mm^2, 1/10-mm deep) was employed as a cell counting device. The chamber was filled using large bore (approx. 1-mm I.D.) Pasteur pipets. At least four 0.1-μl divisions were counted at each filling and at least four separate fillings of the counting chamber were used to calculate the total number of cells per milliliter in the culture. Cells physically attached in groups of four or eight were tallied as a single; these groups were not usually abundant in the culture.

Contamination

Standard plate-count methods were used to determine the number of bacteria present in each CCDA culture. The diluent used was 0.05% NaCl in

0.02 M sodium phosphate buffer, pH 6.0. Aliquots were plated (using a bent glass rod) on an agar medium, which is similar to the algal growth medium and additionally has 0.5% yeast extract, 0.5% dextrose, and 1.7% agar (see recipe below). All plates were incubated at 30°C and counted after 48 hours. Longer incubation was required for detection of some slow-growing cultures.

Recipe for Agar Medium

Constituent	Final Concentration
KNO_3	1.0 g/l
K_2HPO_4	0.085 g/l
KH_2PO_4	0.0675 g/l
$MgSO_4 \cdot 7H_2O$	0.49 g/l
$Ca(NO_3)_2 \cdot 4H_2O$	0.060 g/l
FeEDTA, 2000 ppm, pH 4.3	1.0 ml/l
A_5 micronutrients*	1.0 ml/l
Dextrose	0.5%
Yeast Extract	0.5%
Agar	1.7%

Final pH 6-7

* A_5 micronutrients	Stock Solution (g/l)
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.222
$CuSO_4 \cdot 5H_2O$	0.079
MoO_3 (85%)	0.0177
$NaVO_3$	Pinch

Dissolved in 0.1N H_2SO_4

APPENDIX B

SAMPLE PREPARATION

Elemental Analyzer (Table 4)

The required number of 50-ml beakers were placed in a drying oven at 110°C for 18 hours. The beakers were then placed in a dessicator containing DRIERITE drying agent. After drying, each beaker was weighed and returned to the dessicator.

An aliquot of approximately 52 ml of the nutrient medium was drawn from the reservoir, acidified with 40 μl of concentrated H_2SO_4, and bubbled with argon for 10 minutes to remove CO_2 gas. The volume reduced to 50 ml. The 50 ml of treated nutrient medium was then added to one of the dried, pre-weighed beakers and placed in the drying oven at 110°C for 24 hours.

Approximately 70 ml of the algal suspension were drawn from the culture apparatus and spun in a clinical centrifuge for 20 minutes. An aliquot (52 ml) of the supernatant was taken, treated, and placed in the drying oven following the same procedure as the nutrient sample.

The algal residue was washed with distilled water, spun in the centrifuge, and resuspended in 5 ml of distilled water. This algal suspension was placed on a watch glass and dried in a drying oven at 55°C for 24 hours, after which it was dried for an additional hour at 110°C.

When all samples (nutrient medium, algal supernatant, and algae) were dried, the beakers containing the dried nutrient and supernatant residues were reweighed. These residues, along with that of the algae, were then scraped from the containers, ground with mortar and pestle, and placed in a dessicator with drying agent.

The samples were then analyzed for percent N, C, and H in an elemental analyzer (Perkin-Elmer 240B).

The difference between the element content of the nutrient and that of the supernatant was then computed and compared to that found in the algae.

Elemental Analysis and Ion Chromatography (Table 5)

Three separate aliquots of approximately 30 ml each were taken from the NO_3^- nutrient medium reservoir and filtered (separately) through Whatman 2v filter paper. A 15-ml sample of each filtrate was adjusted to pH 9 to 10 by adding 20 μl of conc. NaOH, making the final volumes 15.02 ml. The pH-adjusted filtrates were placed in plastic tubes with

screw caps and refrigerated for later analysis on a Dionex Model 16 Ion Chromatograph (analysis within 4 hours of preparation).

Similarly, 3 aliquots of the algal suspension were filtered, the residue algae discarded, and 15-ml samples of the filtrate pH-adjusted to 9 to 10 by adding 10 μl conc. NaOH and ~ 5 μl conc. HCl. Final volumes were 15.015 ml. The pH-adjusted filtrates were placed in plastic tubes with screw caps and refrigerated for later analysis on the Dionex (within 4 hours).

Three aliquots of the algal suspension (approximately 14 ml each) were spun in the clinical centrifuge for 10 minutes. The supernatants were discarded and the residue algae washed (and spun) twice with distilled water. Each final algal residue was resuspended in 5 ml distilled H_2O and freeze dried. After freeze drying, the residues were scraped from the drying tubes, placed in plastic snap-cap tubes, and stored in a dessicator before being sent to Galbraith Laboratories, Knoxville, TN, for N and P analysis. Since only 8 mg of algal material were retrieved from each drying tube, the three dried samples were combined for duplicate N and P analysis.

Dry weights for these algae were also done in triplicate.

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16 Abstract

This report discusses studies with algal cultures which relate to closed ecological life support systems (CELSS). A description of a constant cell density apparatus for continuous culture of algae is included. Excretion of algal by-products, and nitrogen utilization and excretion are discussed.

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